177. On the Structure of Podophyllotoxin and the Peltatins

by **A. W. Schrecker** and **J. L. Hartwell.**

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Press & Brun[1]) have recently proposed new structural formulas for podophyllotoxin, α-peltatin and β-peltatin, the biologically active components of Podophyllum peltatum *L.*, and for their "picro" derivatives. Since these suggested structures are greatly at variance with the ones resulting from work in our laboratory[2–7]), a critical discussion of their validity is in order. In the following outline, each of the fractions identified by *Press & Brun* will be dealt with separately.

Podophyllotoxin. Press & Brun's product was recrystallized from benzene-petroleum ether, found to melt at 114–117°, then dried in a vacuum at 50° and analyzed. It is known that podophyllotoxin, when crystallized from benzene, forms a solvate with the empirical formula $2C_{22}H_{22}O_8 \cdot C_6H_6 \cdot 2H_2O$[8])[9]). The solvent of crystallization cannot be removed completely except by vacuum-drying at 100° or higher[2])[8–10]). Analyses carried out by different investigators[2])[9])[10]) with thoroughly dried material, especially with one of the crystalline modifications[2])[9]), agree closely with the theory for $C_{22}H_{22}O_8$. Thus our sample melting at 183–184° gave excellent agreement with the theoretical values not only for the carbon-hydrogen, but also for the methoxyl[2]). Furthermore, all the analyses of the compounds derived directly or indirectly from podophyllotoxin (acetate, halides, apopicropodophyllins, epipodophyllotoxin, desoxypodophyllotoxin, etc.), many of which were confirmed repeatedly by different groups of investigators, agree perfectly with the established formula[2–4])[8–12]), while it is impossible to reconcile them with the formula proposed by *Press & Brun*. Under these circumstances it appears likely that the sample analyzed by these authors still contained benzene, and probably water, of crystallization. This would be consistent with the high carbon and low (even for their own formula) methoxyl values and also with the relatively low optical rotation which they report.

Picropodophyllin. Borsche & Niemann[8]) reported that this compound crystallized from methanol as the solvate, $C_{22}H_{22}O_8 \cdot CH_3OH$, and was dried completely at 118°, while *Späth, Wessely & Kornfeld*[9]) published analyses which agreed perfectly with the theory for the monohydrate before, and for $C_{22}H_{22}O_8$ after, drying at 110°. They observed, in addition, that the monohydrate was formed again from the anhydrous material when allowed to stand in the open. The sample of *Press & Brun*, which was dried at 60°, was probably still partly solvated. It has also been reported[9]) that recrystallization of picropodophyllin sometimes causes partial decomposition instead of effecting purification, as indicated by lowering of the melting point. *Späth*'s best product had m.p. 228° and $[\alpha]_D = +9°$ ($CHCl_3$), while our purest samples melted between 227 and 230° (highest m.p. 231.5–232.5°[2])), with specific rotations ranging around +5°. The value of −5.5° reported by *Press & Brun* suggests the presence of a strongly levorotatory impurity. As in the case of podophyllotoxin, the formula $C_{22}H_{22}O_8$ has repeatedly been substantiated by the analyses of a considerable number of derivatives.

β-Peltatin and *picro-β-peltatin.* It appears evident that the crystalline material isolated from fractions 14–17[1]) is actually podophyllotoxin. This is indicated first by the m.p. of 123–125°, which is that of solvated podophyllotoxin and more than 100° lower than that of β-peltatin[5]). The "picro-β-peltatin" obtained by treatment with ammonia gave a melting point and optical rotation which are about the same as the ones reported by *Press & Brun* for the picropodophyllin which they isolated, but quite at

variance with the corresponding constants of β-peltatin-B[5]). Its failure to react with diazomethane[1]) excludes the presence of a phenolic hydroxyl group, proven to be present in β-peltatin[5])[6]) but absent in picropodophyllin. The UV. spectra of the "β-peltatin" and "picro-β-peltatin" isolated by *Press & Brun* are essentially those of podophyllotoxin and picropodophyllin and differ greatly from that of β-peltatin[5]). It may be noted that the presence of a γ-lactone function in both podophyllotoxin and the peltatins has been demonstrated by the preparation of well-characterized hydroxy acids[2])[6])[8]) and by the characteristic IR. carbonyl stretching frequency[4])[6]). It is difficult to understand why failure to react with diazomethane is taken to indicate the absence of a lactone ring. Adding the yield of fractions 14—17 to that of the preceding ones brings it more in line with the actual proportions of podophyllotoxin[5]) usually present in Podophyllum peltatum. The analysis of *Press & Brun*'s "β-peltatin" can again be explained on the basis of residual solvent of crystallization (in this instance ethyl acetate and water) in incompletely dried podophyllotoxin. Similarly, their "picro-β-peltatin" would consist of picropodophyllin containing residual water and a lesser amount of methanol, in addition to other possible impurities.

It appears surprising that the true β-peltatin was not isolated from any of the fractions. It is most likely that it was present in the mother liquors from the recrystallization of α-peltatin. Determination of the methoxyl content of the various fractions was found in this laboratory[5]) to be the best method for following the separation of α- and β-peltatin.

α-Peltatin and *picro-α-peltatin*. These substances have essentially the same physical properties (melting points, optical rotations, UV. spectra) and are both doubtless identical with our α-peltatin. Examination of the UV. spectra of *Press & Brun*'s α-peltatin and "picro-α-peltatin" indicates that the former was less pure (shallower minimum and greater proportion of impurities absorbing in the region of 310—350 mμ). This agrees with the analytical values which approximate the theory of $C_{21}H_{20}O_8$ more closely in the samples of "picro-α-peltatin". The recrystallization following the treatment of α-peltatin with ammonia evidently produced purification; similarly, fractions 25 and 26 may have contained less contaminating matter than 19 and 20. The failure of α-peltatin to be inverted readily to the dextrorotatory "B"-compound in the presence of ammonia holds also true for β-peltatin[13]) and may be contrasted with the rapid conversion of podophyllotoxin to picropodophyllin under the same conditions. Sodium acetate[5]) or piperidine, however, do effect epimerization readily. The degradation of α-peltatin-B dimethyl ether (proven[6]) to be identical with β-peltatin-B methyl ether) to cotarnic acid and myristicinic acid[7]) has established unequivocally the presence of a methylenedioxy group. Since α- and β-peltatin do not contain the methylenedioxybenzene but the hydroxy-methylenedioxybenzene chromophore, it is not surprising that their UV. spectra do not resemble that of dihydrosafrole. Introduction of a hydroxyl or methoxyl group produces a hypsochromic shift in the maximum. Thus the maximum at 288 mμ ($\log \varepsilon = 3.63$) in safrole[1]) is displaced to 276 mμ ($\log \varepsilon = 3.14$) in myristicin (5-methoxysafrole)[14]).

To summarize, the analyses of podophyllotoxin and picropodophyllin reported by *Press & Brun*[1]) are explained by the presence of residual solvent of crystallization in their samples. Their "β-peltatin" and "picro-β-peltatin" evidently consist of partly solvated podophyllotoxin and picropodophyllin, respectively, while their "picro-α-peltatin" is identical with α-peltatin itself, and their α-peltatin contains traces of impurities. No chemical evidence, such as the preparation of derivatives, has been adduced in proof of their formulas. Thus, there is no reason to abandon the well-established structures of podophyllotoxin[2])[4]), picropodophyllin and the peltatins[7]).

RÉSUMÉ.

Les analyses des podophyllotoxine et picropodophylline publiées par *Press & Brun*[1]) s'expliquent par la présence d'un reste du solvant de cristallisation dans leurs échantillons. Leurs «β-peltatine» et «picro-β-peltatine» ne sont en réalité que de la podophyllotoxine et de la

picropodophylline partiellement solvatées, tandis que leur «picro-α-peltatine» est identique à l'α-peltatine et leur α-peltatine contient des traces d'impuretés. Aucune preuve chimique, telle que la préparation de dérivés, n'est avancée pour soutenir leurs formules. L'examen critique de leurs données et de leurs conclusions montre qu'il n'y a pas lieu d'abandonner des structures chimiques bien établies, en faveur de leurs suggestions.

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[14]) *A. W. Schrecker*, unpublished.

178. Sur la structure de la podophyllotoxine et des peltatines

par **J. Press** et **R. Brun.**

(7 V 54)

Le texte de la note qui précède nous ayant été communiqué grâce à l'obligeance de ses auteurs, nous avons fait faire l'analyse (Laboratoire de M. *A. Peisker-Ritter*, à Brugg) de deux de nos produits: picropodophylline et podophyllotoxine, et ceci dans les conditions indiquées par *A. W. Schrecker & J. L. Hartwell*: séjour de 24 h. à 100° sous 0,001 mm Hg, sur P_2O_5.

Les résultats obtenus pour la *picropodophylline* sont les mêmes que ceux indiqués dans notre publication:

Perte au séchage 2,72%.

4,497 mg subst. ont donné 10,286 mg CO_2 et 2,150 mg H_2O

$C_{23}H_{24}O_9$ (444)	Calculé C 62,16	H 5,44%	Trouvé C 62,42 H 5,35%
$C_{22}H_{22}O_8$ (414)	Calculé „ 63,76	„ 5,31%	